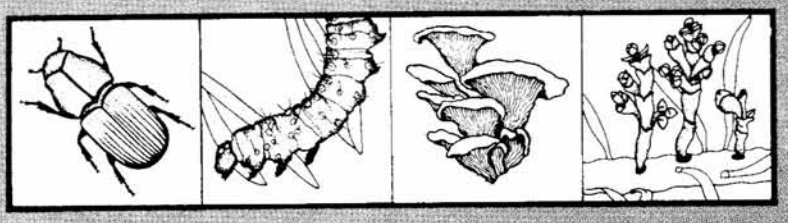
Forest Pest Management



Report 88-7

3450
April 1988

MICROWAVE TREATMENTS TO ERADICATE SEEDBORNE FUNGI ON DOUGLAS-FIR SEED

R. L. James, Plant Pathologist, C.J. Gilligan, Biological Technician, R. K. Dumroese 1/, Research Associate, and D. L. Wenny Associate Professor 1/

INTRODUCTION

Diseases of containerized Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings associated with *Fusarium* spp. are very important at nurseries within the Northern Rocky Mountains. *Fusarium* spp. are common colonizers of Douglas-fir seed (James 1983, James 1984, James 1986b, James et al. 1987a) and infested seed may be major sources of inoculum for infection of containerized seedlings (James 1985b, James 1986a).

Several attempts have been made to eliminate or at least reduce levels of *Fusarium* on conifer seed (James and Genz 1981, James and Genz 1982, James 1987b) including Douglas-fir (Dumroese et al. 1987). Most of these treatments rely on chemical sterilants which are nonspecific and affect all seedcoat organisms. However, past experience has indicated that such treatments may not effectively reduce fungal levels (James 1987a, James et al. 1987a) or may have detrimental effects on seed germination (Edwards and Sutherland 1979) and cause phytotoxicity to young germinants (Cayford and Waldron 1967, Cooley 1983, Lock et al. 1975).

One other approach to reducing seedborne pathogens is hot water treatments (Baker 1962). Hot water treatments have effectively been used on several agricultural crops to reduce or eliminate pathogens on seed while maintaining high levels of germinative capacity and to eliminate phytotoxic reactions (Baker 1956, Neergaard 1977, Walker 1969). A recent innovative approach to hot water treatments is the use of microwaves to heat water to the desired temperature (Lozano et al. 1986). Such a technique can be used to properly regulate exposure time and temperatures and is relatively easy to use commercially.

Since there is no information on responses of conifer seed to hot water treatment by microwaves, an evaluation was conducted to ascertain effects of such treatments on germination and occurrence of certain fungi and bacteria on and within Douglas-fir seed.

1/ Forest Research Nursery, University of Idaho, Moscow.

United States Department of Agriculture | Forest Service | **Northern Region** | P.O. Box 7669 Missoula, Montana 59807

MATERIALS AND METHODS

Seed used in this evaluation was from lot Forest Nursery (FN) from the University of Idaho Research Nursery. Bulk seed was initially rinsed with running tap water for 48 hours, and stratified for 22 days at 3 degrees C. Following stratification, seeds were again rinsed in running tap water, this time for 24 hours. Seeds were then placed in 300 ml distilled water within a glass beaker. The water-seed solution was heated to varying temperatures by exposure to microwaves at the full power setting for different time periods. The microwave oven used was a Kenmore model 99701 with 1,400 watts heating power (2,450 MHz). Water temperatures were recorded before and after microwave treatments. Controls consisted of seeds placed in unheated (20 degrees C) water.

Following microwave treatments, seeds were allowed to cool to room temperature and blotted dry on filter paper. They were then aseptically placed on a selective medium for *Fusarium* (Komada 1975). Two hundred twenty-five seeds per treatment (exposure time and temperature) were plated on the selective medium. Another sample of 25 seeds were randomly selected following each treatment and aseptically dissected to remove their endosperms. Endosperms were then placed on the selective medium. All plates of selective media were incubated at about 22 degrees C under cool fluorescent light for 7 days. Percentages of seed (and endosperms) colonized with *Fusarium*, *Trichoderma*, *Penicillium*, and unidentified bacteria were calculated. Selected isolates of *Fusarium* were obtained and grown on potato dextrose agar and carnation leaf agar for identification using the taxonomic scheme of Nelson et al. (1983).

An additional sample of 150 seeds was randomly collected following microwave treatments for evaluation of effects on germination. Selected seed were placed on absorbant cotton moistened with sterile distilled water within petri dishes, and incubated at 22-24 degrees C under a regime of diurnal cool fluorescent light (12-hour day length). Numbers of germinated seed were determined after 7, 14, 21, and 28 days' incubation. A seed was considered germinated when its radicle was at least the length of its seedcoat.

Data were analyzed using a one-way analysis of variance. Treatment effects on seed germination and organism colonization were located using Tukey's multiple-range comparison test. All percentages underwent arc-sin transformations prior to analyses.

RESULTS AND DISCUSSION

About 3 percent of the untreated seed from the tested seedlot were infested with *Fusarium* (Table 1). Microwave treatments for 60 seconds (water temperature of 43 degrees C) reduced this amount of infection by almost half. Treatments for 90 seconds (55 degrees C) reduced levels even further and no *Fusarium* was detected on seed exposed for 120 seconds (66.5 degrees C) or longer. Increasing time exposure to microwaves resulting in increased water temperatures likewise reduced incidence of both *Trichoderma* and *Penicillium* on seedcoats. However, the amounts of bacteria detected on seedcoats increased with increasing water temperatures, probably because competing fungi were being reduced.

Table 1.--Effects of microwave hot water treatments on occurrence of *Fusarium*, *Trichoderma*, *Penicillium*, and bacteria on seedcoats of Douglas-fir seed.

		Percentage Seedcoat Colonization			
Exposure time (sec.)	Max. water temperature (degrees)	*Fusarium*	*Trichoderma*	*Penicillium*	Bacteria
0	20.0	3.1 A*	98.2 A	71.5 A	0.4 A
60	43.0	1.8 AB	96.9 A	72.0 A	1.8 A
90	55.5	0.4 B	46.7 B	40.4 B	32.4 B
120	66.5	0.0 B	0.4 C	1.8 C	40.4 B
150	77.0	0.0 B	0.4 C	2.2 C	66.2 C
180	88.5	0.0 B	0.0 C	0.0 C	82.2 C

* Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's multiple-range comparison test.

No *Fusarium* was detected within endosperms of the seed sampled (Table 2). Therefore, effects of microwave treatments on these potential pathogens within the seed could not be determined. Usually, a small percentage (less than 1 percent) of Douglas-fir seed have endosperms infected with *Fusarium*; by far most of these fungi occur on the seedcoats (James et al. 1987a). Occurrence of both *Trichoderma* and *Penicillium* within seed endosperms was reduced by microwave hot water treatments. Neither of these fungi were detected after exposures of 120 seconds (66.5 degrees C) or more. The amount of bacteria detected within the endosperm varied among the treatment exposure times.

Table 2.--Effects of microwave hot water treatments on occurrence of *Fusarium*, *Trichoderma*, *Penicillium*, and bacteria within endosperms of Douglas-fir seed.

		Percentage Endosperm Colonization			
Exposure time (sec.)	Max. water temperature (degres C)	*Fusarium*	*Trichoderma*	*Penicillium*	Bacteria
0	20.0	0 A*	28.0 A	24.0 A	28.0 B
60	43.0	0 A	28.0 A	36.0 A	72.0 A
90	55.5	0 A	4.0 B	0 B	8.0 C
120	66.5	0 A	0 B	0 B	56.0 A
150	77.0	0 A	0 B	0 B	72.0 A
180	88.5	0 A	0 B	0 B	8.0 C

* Within each column, means followed by the same capital letter are not significantly different (P = 0.05) using Tukey's multiple-range comparison test.

Effects of microwave hot water treatments on Douglas-fir seed germination are summarized in Table 3. No seed germinated after 120 seconds (66.5 degrees C) of exposure, although germination was not significantly reduced by exposures of 90 seconds (55.5 degrees C) or less. After 7 days' germination of seed exposed to the two lowest treatments (60 seconds = 43 degrees C and 90 seconds = 55.5 degrees C) was somewhat less than the controls (Table 3 and fig. 1). However, after 14 days, germination increased to near control levels. Unfortunately, the exposure time needed to eliminate all *Fusarium* from seed (120 seconds = 66.5 degrees C) also eliminated seed viability. However, the 90 seconds treatment (55.5 degrees C) reduced *Fusarium* levels to almost negligable amounts (0.4 percent) and did not significantly reduce seed germination. Levels of *Trichoderma* were reduced by about half after the 90 seconds exposure. Since these latter organisms are common antagonists against *Fusarium* spp. (Papavizas 1985), reducing their occurrence on seed may not be desirable. This is especially true if *Fusarium* inoculum is introduced into containerized seedlings from sources other than seed, such as containers (James 1987c, James et al. 1987b)) or soil mixes (James 1985a).

Table 3.--Effects of microwave hot water treatments on germination of Douglas-fir seed.

		Percent Germination			
Exposure time (sec.)	Maximum water temperature (degrees C)	7 days	14 days	21 days	28 days
0	20.0	58.0 A*	84.7 A	90.0 A	90.0 A
60	43.0	48.7 A	80.7 A	86.0 A	86.7 A
90	55.5	39.3 A	82.7 A	86.0 A	86.0 A
120	66.5	0 B	0 B	0 B	0 B
150	77.0	0 B	0 B	0 B	0 B
180	88.5	0 B	0 B	0 B	0 B

* Within each column, means followed by the same capital letter are not significantly different (P = 0.05) using Tukey's multiple-range comparison test.

Our data indicate that microwave hot water treatments reduce occurrence of seed fungi including potential pathogens such as *Fusarium*. However, there is a fine line between effectively reducing seed fungi and causing seed death. Treatments somewhere between 60 and 90 seconds (43 and 55.5 degrees C) may be best for practical applications. Additional tests are necessary to locate this thermal "window" more precisely. It is probable that other conifer species will respond differently to hot water treatments. Likewise, other Douglas-fir seedlots may respond differently. Larger quantities of seed treated at one time may also react differently. Additional tests will be required to elucidate these possible differences.

Treatments of agricultural seeds such as soybeans using vegetable oils, such as sunflower, soybean and maize oils as the medium for heat treatment instead of water, have been effective (Ryndji et al. 1987, Zinnen and Sinclair 1982). The major advantage of vegetable oils over water is reduced seed imbibition of the heated medium and resulting toxicity to the embryo. There is currently no information available as to the responses of conifer seeds to such treatments, but evaluations may be beneficial because of the toxicity of hot water (66.5 degrees C and above) to Douglas-fir seed.

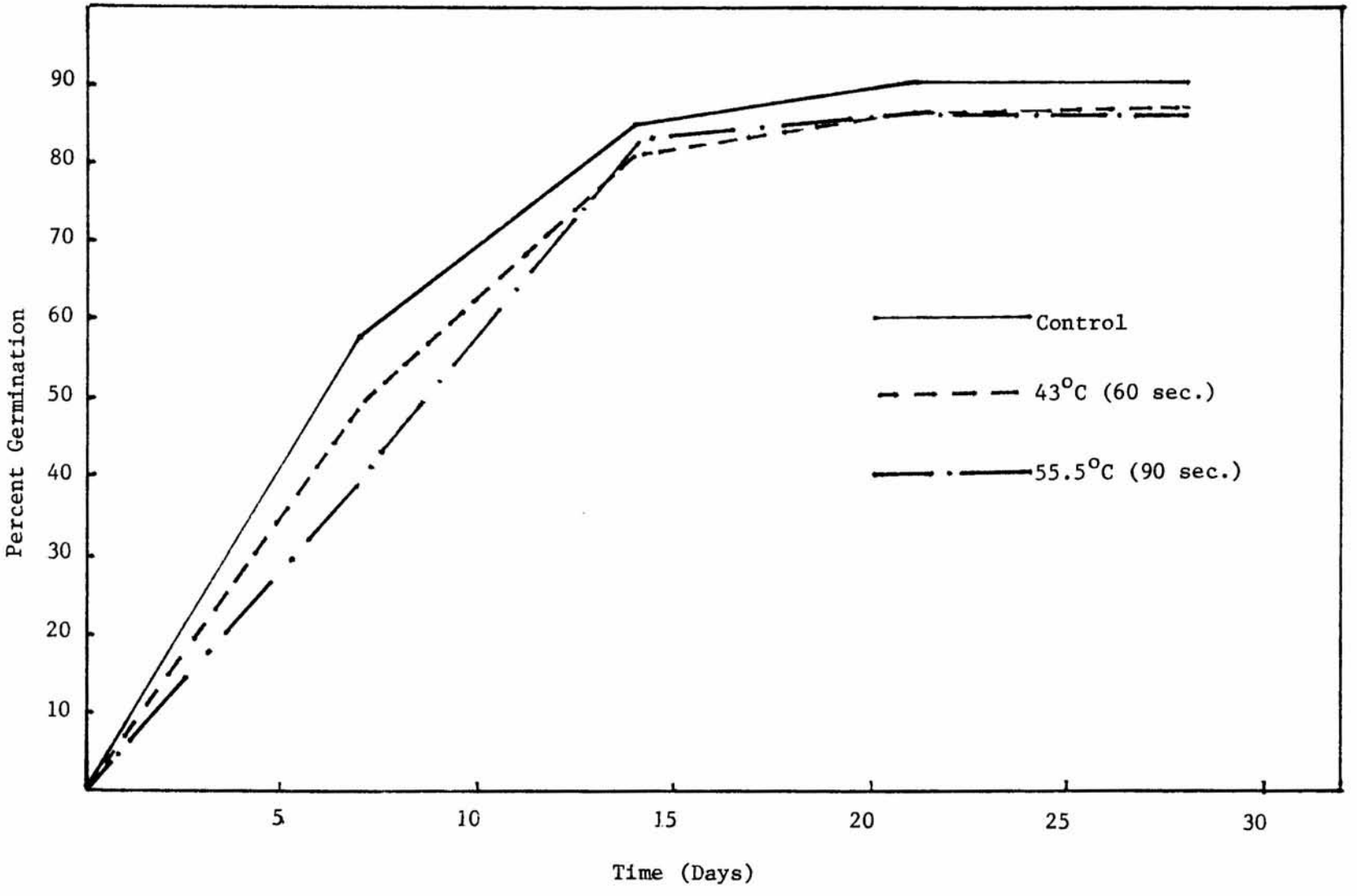


Figure 1. Germination over time of Douglas-fir seed undergoing microwave hot water treatments.

LITERATURE CITED

Baker, K. F. 1956. Development and production of pathogen-free seed of three ornamental plants. Plant Dis. Reptr. Suppl. 238: 68-71.

Baker, K. F. 1962. Thermotherapy of planting material. Phytopathology 52: 1244-1255.

Cayford, J. H. and R. M. Waldron. 1967. Effects of captan on germination of white spruce, jack and red pine seed. For. Chron. 43: 381-384.

Cooley, S. J. 1983. Fungicide trials on sugar pine at a southern Oregon nursery. Tree Planters' Notes. 34(3): 15-18.

Dumroese, R. K., R. L. James, and D. L. Wenny. 1987. Douglas-fir seed treatments: effects on seed germination and seedborne organisms. USDA For. Serv., Northern Region (in preparation).

Edwards, D. G. W. and J. R. Sutherland. 1979. Hydrogen peroxide treatment of *Abies* seeds. Can. For. Serv. Bi-Monthly Res. Notes 35: 3-4.

James, R. L. 1983. Occurrence of *Fusarium* on Douglas-fir seed from the Coeur d'Alene Nursery. USDA For. Serv., Northern Region. 11 p.

James, R. L. 1984. Fungi colonizing Douglas-fir seed at the Champion Timberlands Nursery, Plains, Montana. USDA For. Serv., Northern Region. Rept. 84-13. 3 p.

James, R. L. 1985a. Diseases associated with containerized seedling soil mixes. Tree Planters' Notes 36(2): 3-5.

James, R. L. 1985b. Pathogenic *Fusarium* on spruce seed from the Towner Nursery, North Dakota. USDA For. Serv., Northern Region. Rept. 85-23. 9 p.

James, R. L. 1986a. Diseases of conifer seedlings caused by seed-borne *Fusarium* species. *In*: Shearer, R. C. (compiler). Proceedings: Conifer Tree Seed in the Inland Mountain West Symposium. USDA For. Serv., Gen. Tech. Rept. INT-203. pp. 267-271.

James, R. L. 1986b. Occurrence of *Fusarium* on Douglas-fir seed and containerized seedlings at the Plum Creek Nursery, Pablo, Montana. USDA For. Serv., Northern Region. Rept. 86-4. 10 p.

James, R. L. 1987a. Fungi on bleach-treated western white pine seed, Raintree Nursery, Libby, Montana. USDA For. Serv., Northern Region. 1 p.

James, R. L. 1987b. Occurrence of *Fusarium* on conifer tree seed from Northern Rocky Mountain nurseries. *In*: Landis, T. D. (Tch. Coord.). Proceedings: Combined Western Forest Nursery Council and Intermountain Nursery Association Meeting. USDA For. Serv., Gen. Tech. Rept. RM-137. pp. 109-114.

James, R. L. 1987c. Occurrence of *Fusarium* within styroblock containers - Plum Creek Nursery, Pablo, Montana (Preliminary Report). USDA For. Serv., Northern Region. 2 p.

James, R. L. and D. Genz. 1981. Ponderosa pine seed treatments: effects on seed germination and disease incidence. USDA For. Serv., Northern Region. Rept. 81-16. 13 p.

James, R. L. and D. Genz. 1982. Evaluation of fungal populations on ponderosa pine seed. USDA For. Serv., Northern Region. Rept. 82-22. 21 p.

James, R. L., R. K. Dumroese, D. L. Wenny, J. F. Myers, and C. J. Gilligan. 1987a. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 1. Seed and seedling infection, symptom production, and disease progression. USDA For. Serv., Northern Region (in preparation).

James, R. L., C. J. Gilligan, and V. Reedy. 1987b. Evaluation of root diseases of containerized conifer seedlings at the Champion Timberlands Nursery, Plains, Montana. USDA For. Serv., Northern Region (in preparation).

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Protec. Res. 8: 114-125.

Lock, W., J. R. Sutherland and L. J. Sluggett. 1975. Fungicide treatment of seeds for damping-off control in British Columbia forest nurseries. Tree Planters' Notes 26(3): 16-18

Lozano, J. C., R. Laberry and A. Bermudez. 1986. Microwave treatment to eradicate seed-borne pathogens in cassava tree seed. Jour. of Phytopathology 117: 1-8.

Neergaard, P. 1977. Seed pathology. John Wiley & Sons, New York. 1187 p.

Nelson, P. E., T. A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State Univ. Press, University Park. 193 p.

Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potaential for biocontrol. Ann. Rev. Phytopathol. 23: 23-54.

Ryndji, M. M., J. B. Sinclair, and T. Singh. 1987. Soybean seed themotherapy with heated vegetable oils. Plant Disease 71: 213-216.

Walker, J. C. 1969. Plant pathology (3rd edition). McGraw-Hill Book Company, New York. 819 p.

Zinnen, T. M. and J. B. Sinclair. 1982. Thermotherapy of soybean seeds to control seedborne fungi. Phytopathology 72: 831-834.